

Human Placental Assay Quick Response Task Results.

Quick Response Task #1.

See attached report (Report_revised_11_6.wpd)

Quick Response Task #2.

For this work, the protein concentration was fixed at 0.0125 mg/mL, the [3H]ASDN concentration was 100 nM and NADPH concentration was 0.3 mM. Some tubes also contained 100 nM 4-OH ASDN as an inhibitor. The response of the assay to various incubation times (5-30 min) was explored. The results are presented in Table 1 and Figure 1. The aromatase activities measured at each time point in the presence and the absence of the inhibitor are presented in Figure 2. In order to test whether NADPH became limiting during the course of the reaction, an experiment in which an additional aliquot (0.3 mM final concentration) of NADPH was added midway through a 30 minute incubation period. Other runs were conducted in which a single aliquot of NADPH (0.3 mM) was added to the reaction at the beginning of the incubation period. Table 2 shows a comparison of the activities measured in the NADPH supplemented and unsupplemented cases.

Table 1. Human Placental Assay: Response of Activity to Incubation Time

Time (min)	Product formation rate (nmol/mg)				Aromatase activity (nmol/mg/min)				% substrate conversion (complete assay)	% Inhibition		
	Total activity		Inhibited		Total activity		Inhibited					
	average	SD	average	SD	average	SD	average	SD				
5	0.2124	0.0130	0.0981	0.0008	0.0425	0.0026	0.0196	0.0002	2.73	53.8		
10	0.4025	0.0090	0.1245	0.0005	0.0403	0.0009	0.0124	0.0000	5.11	69.1		
15	0.8039	0.0108	0.1890	0.0053	0.0536	0.0007	0.0126	0.0004	10.16	76.5		
20	1.0987	0.0345	0.2054	0.0029	0.0549	0.0017	0.0103	0.0001	13.84	81.3		
30	1.3922	0.0138	0.2085	0.0020	0.0464	0.0005	0.0070	0.0001	17.53	85.0		

Table 2: Comparison of Aromatase Activity with and without NADPH Supplementation

	Aromatase activity (nmol/mg/min)			
	Total activity		Inhibited	
	average	SD	average	SD
Unsupplemented	0.0464	0.0005	0.0070	0.0001
Supplemented	0.0445	0.0007	0.0071	0.0001

Quick Response #3

In this task, the assay conditions were set at 100 nM [³H]ASDN, 0.3 mM NADPH, 0.0125 mg/mL protein and a 15 min incubation time. The response of the aromatase assay to varying concentrations of the inhibitor 4-OH ASDN was determined. The response curve and IC₅₀ data are presented below. The calculated IC₅₀, 42 nM, falls within the 30-50 nM range reported in the protocol.

Figure 3: Calculation of IC₅₀ for 4-OH ASDN in the Human Placental Aromatase Assay

The aromatase activities measured for the human placental assay over the three days of experimentation are compared in Table 3 and Figure 4.

Table 3: Human Placental Aromatase Activity for Quick Response Tasks 1-3.

Experiment	Aromatase activity (nmol/mg/min)			
	Total activity		Inhibited	
	average	SD	average	SD
QR1	0.0524	0.0028	0.0119	0.0009
QR2	0.0536	0.0007	0.0126	0.0004
QR3	0.0543	0.0022	0.0148	0.0003

Figure 4 Human Placental Aromatase Activity for Quick Response Tasks 1-3..

Human Recombinant Assay Quick Response Task Results

Quick Response Task #4

For this work, the protein concentration was fixed at 0.005 mg/mL, the [³H]ASDN concentration was 100 nM and NADPH concentration was 0.3 mM. Some tubes also contained 100 nM 4-OH ASDN as an inhibitor. The response of the assay to various incubation times (5-30 min) was explored. The results are presented in Table 4 and Figure 5. The aromatase activities measured at each time point in the presence and the absence of the inhibitor are presented in Figure 6. In order to test whether NADPH became limiting during the course of the reaction, an experiment in which an additional aliquot (0.3 mM final concentration) of NADPH was added midway through a 30 minute incubation period. Other runs were conducted in which a single aliquot of NADPH (0.3 mM) was added to the reaction at the beginning of the incubation period. Table 5 shows a comparison of the activities measured in the NADPH supplemented and unsupplemented cases.

Table 4 Human Recombinant Assay: Response of Activity to Incubation Time

Time (min)	Product formation rate (nmol/mg)				Aromatase activity (nmol/mg/min)				% substrate conversion (complete assay)	% Inhibition		
	Total activity		Inhibited		Total activity		Inhibited					
	average	SD	average	SD	average	SD	average	SD				
5	1.3972	0.1108	0.6196	0.0505	0.2794	0.0222	0.1239	0.0101	6.90	55.7		
10	2.4712	0.0733	0.8665	0.0373	0.2471	0.0073	0.0866	0.0037	12.16	64.9		
15	5.0069	0.0999	1.4029	0.0234	0.3338	0.0067	0.0935	0.0016	24.56	72.0		
20	6.7141	0.0906	1.5180	0.0331	0.3357	0.0045	0.0759	0.0017	32.91	77.4		
30	8.4794	0.1021	1.6452	0.0130	0.2826	0.0034	0.0548	0.0004	41.55	80.6		

Table 5 Comparison of Aromatase Activity with and without NADPH Supplementation

	Aromatase activity (nmol/mg/min)			
	Total activity		Inhibited	
	average	SD	average	SD
Unsupplemented	0.2826	0.0034	0.0548	0.0004
Supplemented	0.2779	0.0062	0.0543	0.0000

Quick Response Task #5

In this task, the assay conditions were set at 100 nM [³H]ASDN, 0.3 mM NADPH, 0.004 mg/mL protein and a 15 min incubation time. The response of the aromatase assay to varying concentrations of the inhibitor 4-OH ASDN was determined. The response curve and IC₅₀ data are presented in Figure 7. The calculated IC₅₀, 25 nM, falls near the 30-50 nM range reported in the protocol.

Figure 7: Calculation of IC₅₀ for 4-OH ASDN in the Human Recombinant Aromatase Assay

The optimized assay conditions for the human placental and human recombinant aromatase assay are summarized in Table 6 below. These conditions were used in the subsequent variance assessment and the test substance phases of the project.

Table 6 Optimized Assay Conditions for Aromatase.

Assay factor (units)	Assay Type	
	Human Placental	Human Recombinant
Microsomal Protein (mg/mL)	0.0125	0.004
NADPH (mM)	0.3	0.3
[³ H]ASDN (nM)	100	100
Incubation Time (min)	15	15

Variance testing of the optimized assay

The optimized conditions for the human placental and recombinant assays were used in the variability assessment of the assays. Three technicians conducted each assay independently over three days. The activity results are presented in Tables 7 and 8 for the placental and recombinant assays, respectively. Statistical analysis of these results will be forthcoming.

Table 7 Placental Aromatase Activities Measured by Three Technicians over Three Days using the Optimized Assay Conditions.

Technician	Day	Replicate	Activity
1	3	1	0.0342
		2	0.0351
		3	0.0337
1	4	1	0.0376
		2	0.0392
		3	0.0473
1	5	1	0.0565
		2	0.0529
		3	0.0560
2	3	1	0.0750
		2	0.0723
		3	0.0714
2	4	1	0.0600
		2	0.0598
		3	0.0581
2	5	1	0.0181
		2	0.0185
		3	0.0178
3	3	1	0.0846
		2	0.0896
		3	0.0845
3	4	1	0.0800
		2	0.0826
		3	0.0818
3	5	1	0.0643
		2	0.0650
		3	0.0664

Table 8. Recombinant Aromatase Activities Measured by Three Technicians over Three Days using the Optimized Assay Conditions.

Technician	Day	Replicate	Activity
1	3	1	0.2623
		2	0.2499
		3	0.2498
1	4	1	0.2124
		2	0.2094
		3	0.2118
1	5	1	0.3260
		2	0.2888
		3	0.3110
2	3	1	0.3792
		2	0.3698
		3	0.3461
2	4	1	0.2996
		2	0.2950
		3	0.3196
2	5	1	0.0837
		2	0.0868
		3	0.0914
3	3	1	0.2759
		2	0.2757
		3	0.2746
3	4	1	0.4364
		2	0.4257
		3	0.4182
3	5	1	0.4096
		2	0.3972
		3	0.3897

Determination of the Response of the Optimized assay to Selected test Substances

Test substance groupings (Table 9) were made based on solubility and whether the chemicals were expected so be inhibitors. Some inhibitors and some non-inhibitors were included in each group. Group 3 consisted of some chemicals that were soluble in EtOH and some that were soluble in DMSO. For this group only, a doubled set of positive and negative controls (quadruplicate each with DMSO and EtOH) were run.

Control activities were calculated and comparisons were made as described in protocol Section 4.4.3.1. Positive and negative control mean activities and standard deviations are presented in Table 10 and graphically in Figure 9. Results of ANOVA for the control data are presented in Table 11.

Table 9. Test Substance Groupings, Target Concentrations and Solvents

Test Group	Test Substance	Levels	Target Concentrations (M)	Solvent
1	econazole	8	10 ⁻⁶ ; 10 ⁻⁷ ; 2.5 and 5 x 10 ⁻⁷ ; 10 ⁻⁸ ; 2.5 and 5 x 10 ⁻⁸ ; 10 ⁻⁹	DMSO
	genistein	8	10 ⁻³ ; 10 ⁻⁴ ; 2.5 and 5 x 10 ⁻⁴ ; 10 ⁻⁵ ; 2.5 and 5 x 10 ⁻⁵ ; 10 ⁻⁶	DMSO
	atrazine	7	10 ⁻³ to 10 ⁻⁹	DMSO
	bis-(2-ethylhexyl)phthalate	7	10 ⁻³ to 10 ⁻⁹	DMSO
2	aminoglutethimide	8	10 ⁻³ ; 10 ⁻⁴ ; 10 ⁻⁵ ; 2.5 and 5 x 10 ⁻⁵ ; 10 ⁻⁶ ; 10 ⁻⁷ ; 10 ⁻⁸	DMSO
	chrysins	8	10 ⁻³ ; 10 ⁻⁴ ; 10 ⁻⁵ ; 2.5 and 5 x 10 ⁻⁵ ; 10 ⁻⁶ ; 10 ⁻⁷ ; 10 ⁻⁸	DMSO
	nonylphenol	7	10 ⁻³ to 10 ⁻⁹	DMSO
	lindane	7	10 ⁻³ to 10 ⁻⁹	DMSO
3	4-OH ASDN	8	10 ⁻⁶ ; 10 ⁻⁷ ; 2.5 and 5 x 10 ⁻⁷ ; 10 ⁻⁸ ; 2.5 and 5 x 10 ⁻⁸ ; 10 ⁻⁹	EtOH
	ketoconazole	8	0.8 x 10 ⁻³ ; 10 ⁻⁴ ; 2.5 and 5 x 10 ⁻⁴ ; 10 ⁻⁵ ; 2.5 and 5 x 10 ⁻⁵ ; 10 ⁻⁶	EtOH
	dibenz (a,h)anthracene	6	10 ⁻⁴ to 10 ⁻⁹	DMSO

Table 10. Mean and Standard Deviations of Control Activities

Microsome	Control			Portion of Batch	Batch (Day) 1		Batch (Day) 2		Batch (Day) 3		Batch (Day) 4	
		Type	Group		Mean	Std Dev						
Placental	Negative	1		Begin	-0.0013	0.0000	-0.0004	0.0006	0.0000	0.0000	0.0000	0.0000
Placental	Negative	1		End	0.0013	0.0008	0.0004	0.0001	0.0000	0.0000	0.0000	0.0001
Placental	Negative	2		Begin	-0.0026	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Placental	Negative	2		End	0.0026	0.0076	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000
Placental	Negative	3-DMSO		Begin	-0.0003	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Placental	Negative	3-DMSO		End	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
Placental	Negative	3-EtOH		Begin	-0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001
Placental	Negative	3-EtOH		End	0.0002	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000
Placental	Positive	1		Begin	0.0647	0.0021	0.2520	0.0016	0.0695	0.0001	0.0629	0.0009
Placental	Positive	1		End	0.0598	0.0020	0.2444	0.0063	0.0646	0.0003	0.0610	0.0015
Placental	Positive	2		Begin	0.2655	0.0005	0.0715	0.0016	0.0549	0.0005	0.0504	0.0010
Placental	Positive	2		End	0.2657	0.0142	0.0666	0.0012	0.0538	0.0005	0.0474	0.0016
Placental	Positive	3-DMSO		Begin	0.0917	0.0028	0.0647	0.0022	0.0519	0.0003	0.0606	0.0002
Placental	Positive	3-DMSO		End	0.0862	0.0011	0.0615	0.0021	0.0493	0.0008	0.0577	0.0010
Placental	Positive	3-EtOH		Begin	0.0863	0.0015	0.0670	0.0009	0.0499	0.0014	0.0610	0.0008
Placental	Positive	3-EtOH		End	0.0843	0.0005	0.0645	0.0029	0.0471	0.0006	0.0566	0.0009

Microsome	Control	Portion of Batch			Batch (Day) 1		Batch (Day) 2		Batch (Day) 3		Batch (Day) 4	
		Type	Type	Group	Mean	Std Dev						
Recombinant	Negative	1		Begin	0.0000	0.0003	0.0000	0.0001	0.0000	0.0001	0.0000	0.0001
Recombinant	Negative	1		End	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0002
Recombinant	Negative	2		Begin	-0.0001	0.0001	0.0000	0.0000	-0.0001	0.0000	0.0000	0.0000
Recombinant	Negative	2		End	0.0001	0.0001	0.0000	0.0000	0.0001	0.0002	0.0000	0.0001
Recombinant	Negative	3-DMSO		Begin	0.0000	0.0001	0.0000	0.0000	0.0001	0.0000	0.0002	0.0003
Recombinant	Negative	3-DMSO		End	0.0000	0.0001	0.0000	0.0002	-0.0001	0.0000	-0.0002	0.0001
Recombinant	Negative	3-EtOH		Begin	0.0001	0.0001	0.0000	0.0001	0.0001	0.0001	0.0001	0.0000
Recombinant	Negative	3-EtOH		End	-0.0001	0.0002	0.0000	0.0001	-0.0001	0.0001	-0.0001	0.0001
Recombinant	Positive	1		Begin	0.5545	0.0023	0.8213	0.0478	0.3260	0.0116	0.3985	0.0040
Recombinant	Positive	1		End	0.5483	0.0181	0.7002	0.0547	0.2878	0.0213	0.3781	0.0259
Recombinant	Positive	2		Begin	0.7927	0.0510	0.5220	0.0028	0.3298	0.0050	0.3609	0.0393
Recombinant	Positive	2		End	0.7146	0.0019	0.4973	0.0249	0.3013	0.0131	0.3092	0.0001
Recombinant	Positive	3-DMSO		Begin	0.8042	0.0033	0.3805	0.0185	0.3077	0.0141	0.4126	0.0053
Recombinant	Positive	3-DMSO		End	0.7704	0.0141	0.3627	0.0033	0.3174	0.0084	0.3822	0.0066
Recombinant	Positive	3-EtOH		Begin	0.8588	0.1277	0.3546	0.0207	0.2925	0.0055	0.3346	0.0051
Recombinant	Positive	3-EtOH		End	0.7218	0.0467	0.3102	0.0121	0.2455	0.0180	0.2992	0.0154

For Figure 9 see file Figure 9.wpd.

Table 11. ANOVA Results for Control Data

Microsome Type	Control Type	Source	DF	Type I SS	Mean Square	F Value	Pr > F
Placental	Negative	Group	3	8.634E-36	2.878E-36	0.00	1.0000
Placental	Negative	Batch(Group)	12	3.242E-35	2.702E-36	0.00	1.0000
Placental	Negative	Portion	1	5.868E-06	5.868E-06	2.96	0.0925
Placental	Negative	Group*Portion	3	4.030E-06	1.343E-06	0.68	0.5708
Placental	Positive	Group	3	3.189E-02	1.063E-02	1374.50	<.0001
Placental	Positive	Batch(Group)	12	2.392E-01	1.993E-02	2577.56	<.0001
Placental	Positive	Portion	1	1.830E-04	1.830E-04	23.66	<.0001
Placental	Positive	Group*Portion	3	1.518E-05	5.059E-06	0.65	0.5846
Recombinant	Negative	Group	3	2.660E-37	8.865E-38	0.00	1.0000
Recombinant	Negative	Batch(Group)	12	1.652E-36	1.376E-37	0.00	1.0000
Recombinant	Negative	Portion	1	8.330E-09	8.330E-09	0.59	0.4481
Recombinant	Negative	Group*Portion	3	1.222E-07	4.072E-08	2.86	0.0474
Recombinant	Positive	Group	3	4.673E-02	1.558E-02	13.67	<.0001
Recombinant	Positive	Batch(Group)	12	2.250E+00	1.875E-01	164.56	<.0001
Recombinant	Positive	Portion	1	3.107E-02	3.107E-02	27.26	<.0001
Recombinant	Positive	Group*Portion	3	4.644E-03	1.548E-03	1.36	0.2680

Aromatase activities in the presence of the various test substances were converted to percent of full activity and the data was fitted to the model:

$$\text{pcnt_activity} = 100 / [(c/\text{conc})^h + 1]$$

where

pcnt_activity = % full activity

c = IC50

h = slope at IC50

conc = concentration of inhibitor.

The aromatase activity and percent of full activity data are presented in Tables 12 and 13 for the placental and recombinant assay, respectively.. The response curves are presented in Figures 10 and 11 for the placental and recombinant assay, respectively.

Table 12. Placental Aromatase Activity in the Presence of Various Test Chemicals.

Chemical	Level	Concentration (M)	Log(concen- tration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Econazole	1	1.00E-06	-6.00	-0.0012	-1.88	-0.0018	-0.73	0.0004	0.56	0.0001	0.18
Econazole	2	5.00E-07	-6.30	-0.0012	-1.88	-0.0017	-0.69	0.0005	0.68	0.0002	0.35
Econazole	3	2.50E-07	-6.60	-0.0008	-1.34	-0.0015	-0.61	0.0008	1.18	0.0005	0.80
Econazole	4	1.00E-07	-7.00	-0.0001	-0.24	-0.0005	-0.21	0.0018	2.73	0.0014	2.04
Econazole	5	5.00E-08	-7.30	0.0010	1.61	0.0007	0.30	0.0034	5.10	0.0031	4.60
Econazole	6	2.50E-08	-7.60	0.0032	5.17	0.0038	1.52	0.0063	9.33	0.0050	7.53
Econazole	7	1.00E-08	-8.00	0.0089	14.23	0.0115	4.65	0.0134	19.98	0.0116	17.37
Econazole	8	1.00E-09	-9.00	0.0422	67.83	0.0941	37.91	0.0506	75.49	0.0456	68.08
Genistein	1	1.00E-03	-3.00	0.0414	66.55	0.0887	35.75	0.0508	75.85	0.0394	58.76
Genistein	2	5.00E-04	-3.30	0.0431	69.18	0.1123	45.26	0.0565	84.33	0.0467	69.66
Genistein	3	2.50E-04	-3.60	0.0471	75.64	0.1323	53.30	0.0569	84.94	0.0500	74.62
Genistein	4	1.00E-04	-4.00	0.0564	90.65	0.1987	80.08	0.0605	90.29	0.0551	82.20
Genistein	5	5.00E-05	-4.30	0.0591	94.95	0.2305	92.90	0.0685	102.27	0.0618	92.26
Genistein	6	2.50E-05	-4.60	0.0589	94.55	0.2463	99.25	0.0705	105.14	0.0609	90.85
Genistein	7	1.00E-05	-5.00	0.0603	96.81	0.2502	100.83	0.0682	101.75	0.0624	93.07
Genistein	8	1.00E-06	-6.00	0.0606	97.29	0.2475	99.73	0.0674	100.50	0.0596	88.96

Chemical	Level	Concentration (M)	Log(concentration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Atrazine	1	1.00E-03	-3.00	0.0560	89.92	0.1550	62.47	0.0632	94.23	0.0555	82.81
Atrazine	2	1.00E-04	-4.00	0.0607	97.51	0.2214	89.21	0.0674	100.56	0.0642	95.76
Atrazine	3	1.00E-05	-5.00	0.0624	100.20	0.2527	101.83	0.0676	100.85	0.0610	90.98
Atrazine	4	1.00E-06	-6.00	0.0621	99.77	0.2548	102.68	0.0656	97.83	0.0640	95.49
Atrazine	5	1.00E-07	-7.00	0.0617	99.03	0.2539	102.33	0.0693	103.42	0.0635	94.69
Atrazine	6	1.00E-08	-8.00	0.0621	99.74	0.2491	100.38	0.0682	101.71	0.0616	91.97
Atrazine	7	1.00E-09	-9.00	0.0613	98.41	0.2501	100.78	0.0673	100.37	0.0611	91.16
bis(2-ethylhexyl)phthalate	1	1.00E-03	-3.00	0.0528	84.87	0.2248	90.59	0.0638	95.26	0.0596	88.93
bis(2-ethylhexyl)phthalate	2	1.00E-04	-4.00	0.0598	95.98	0.2478	99.85	0.0665	99.28	0.0608	90.74
bis(2-ethylhexyl)phthalate	3	1.00E-05	-5.00	0.0165	26.47	0.2536	102.21	0.0674	100.56	0.0639	95.38
bis(2-ethylhexyl)phthalate	4	1.00E-06	-6.00	0.0640	102.83	0.2610	105.15	0.0673	100.46	0.0631	94.09
bis(2-ethylhexyl)phthalate	5	1.00E-07	-7.00	0.0633	101.67	0.2474	99.68	0.0674	100.56	0.0625	93.25
bis(2-ethylhexyl)phthalate	6	1.00E-08	-8.00	0.0631	101.38	0.2596	104.61	0.0651	97.16	0.0607	90.61
bis(2-ethylhexyl)phthalate	7	1.00E-09	-9.00	0.0614	98.58	0.2426	97.77	0.0639	95.27	0.0598	89.18
Aminoglutethimide	1	1.00E-03	-3.00	-0.0094	-3.55	0.0005	0.75	0.0004	0.68	0.0003	0.65
Aminoglutethimide	2	1.00E-04	-4.00	-0.0036	-1.37	0.0048	6.98	0.0038	7.07	0.0033	6.79
Aminoglutethimide	3	5.00E-05	-4.30	0.0012	0.45	0.0087	12.63	0.0069	12.65	0.0066	13.43
Aminoglutethimide	4	2.50E-05	-4.60	0.0104	3.91	0.0179	25.97	0.0125	23.07	0.0110	22.57
Aminoglutethimide	5	1.00E-05	-5.00	0.0417	15.68	0.0304	44.04	0.0234	43.07	0.0229	46.88
Aminoglutethimide	6	1.00E-06	-6.00	0.1817	68.39	0.0596	86.33	0.0477	87.84	0.0461	94.26
Aminoglutethimide	7	1.00E-07	-7.00	0.2568	96.67	0.0693	100.39	0.0548	100.86	0.0514	105.12
Aminoglutethimide	8	1.00E-08	-8.00	0.2640	99.39	0.0700	101.29	0.0557	102.43	0.0491	100.44

Chemical	Level	Concentration (M)	Log(concentration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Chrysin	1	1.00E-03	-3.00	0.0231	8.71	0.0223	32.22	0.0181	33.25	0.0160	32.64
Chrysin	2	1.00E-04	-4.00	0.0256	9.62	0.0239	34.53	0.0195	35.91	0.0178	36.45
Chrysin	3	5.00E-05	-4.30	0.0167	6.30	0.0142	20.60	0.0078	14.34	0.0126	25.87
Chrysin	4	2.50E-05	-4.60	0.0075	2.84	0.0133	19.30	0.0104	19.22	0.0088	17.99
Chrysin	5	1.00E-05	-5.00	0.0292	11.01	0.0254	36.71	0.0208	38.29	0.0179	36.67
Chrysin	6	1.00E-06	-6.00	0.1660	62.50	0.0593	85.90	0.0499	91.83	0.0418	85.42
Chrysin	7	1.00E-07	-7.00	0.2548	95.91	0.0667	96.56	0.0565	103.99	0.0477	97.56
Chrysin	8	1.00E-08	-8.00	0.2717	102.28	0.0677	97.97	0.0553	101.79	0.0482	98.66
Nonylphenol	1	1.00E-03	-3.00	-0.0098	-3.68	0.0000	0.06	0.0000	0.09	-0.0001	-0.15
Nonylphenol	2	1.00E-04	-4.00	-0.0001	-0.02	0.0022	3.15	0.0020	3.62	0.0018	3.59
Nonylphenol	3	1.00E-05	-5.00	0.1742	65.59	0.0594	85.99	0.0465	85.53	0.0429	87.82
Nonylphenol	4	1.00E-06	-6.00	0.2572	96.83	0.0694	100.42	0.0578	106.43	0.0485	99.29
Nonylphenol	5	1.00E-07	-7.00	0.2671	100.55	0.0669	96.93	0.0551	101.42	0.0473	96.72
Nonylphenol	6	1.00E-08	-8.00	0.2803	105.53	0.0689	99.78	0.0580	106.76	0.0479	97.92
Nonylphenol	7	1.00E-09	-9.00	0.2616	98.47	0.0690	99.88	0.0535	98.41	0.0479	97.92
Lindane	1	1.00E-03	-3.00	0.2126	80.02	0.0611	88.50	0.0504	92.75	0.0378	77.37
Lindane	2	1.00E-04	-4.00	0.2127	80.09	0.0662	95.82	0.0515	94.73	0.0442	90.41
Lindane	3	1.00E-05	-5.00	0.2577	97.02	0.0655	94.87	0.0549	101.00	0.0478	97.76
Lindane	4	1.00E-06	-6.00	0.2688	101.19	0.0666	96.47	0.0574	105.59	0.0472	96.61
Lindane	5	1.00E-07	-7.00	0.2673	100.65	0.0656	94.91	0.0563	103.58	0.0448	91.73
Lindane	6	1.00E-08	-8.00	0.2719	102.34	0.0669	96.85	0.0565	103.92	0.0445	91.13
Lindane	7	1.00E-09	-9.00	0.2622	98.73	0.0672	97.36	0.0549	100.96	0.0477	97.55

Chemical	Level	Concentration (M)	Log(concentration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Dibenz(a, h)anthracene	1	1.00E-04	-4.00	0.0810	91.10	0.0614	97.27	0.0492	97.21	0.0585	99.02
Dibenz(a, h)anthracene	2	1.00E-05	-5.00	0.0902	101.36	0.0646	102.41	0.0511	101.02	0.0581	98.22
Dibenz(a, h)anthracene	3	1.00E-06	-6.00	0.0873	98.12	0.0646	102.42	0.0499	98.50	0.0604	102.10
Dibenz(a, h)anthracene	4	1.00E-07	-7.00	0.0874	98.20	0.0643	101.96	0.0509	100.62	0.0596	100.85
Dibenz(a, h)anthracene	5	1.00E-08	-8.00	0.0891	100.14	0.0644	102.04	0.0507	100.09	0.0596	100.77
Dibenz(a, h)anthracene	6	1.00E-09	-9.00	0.0865	97.23	0.0635	100.70	0.0498	98.37	0.0580	98.08
Ketoconazole	1	8.00E-04	-3.10	-0.0001	-0.09	0.0001	0.19	0.0000	0.07	0.0002	0.34
Ketoconazole	2	5.00E-04	-3.30	0.0016	1.93	0.0004	0.65	0.0002	0.33	0.0003	0.58
Ketoconazole	3	2.50E-04	-3.60	0.0019	2.21	0.0010	1.46	0.0005	1.04	0.0009	1.55
Ketoconazole	4	1.00E-04	-4.00	0.0081	9.48	0.0064	9.69	0.0042	8.56	0.0049	8.35
Ketoconazole	5	5.00E-05	-4.30	0.0143	16.80	0.0116	17.69	0.0078	16.06	0.0111	18.81
Ketoconazole	6	2.50E-05	-4.60	0.0269	31.50	0.0211	32.08	0.0146	30.05	0.0176	29.84
Ketoconazole	7	1.00E-05	-5.00	0.0461	54.05	0.0347	52.80	0.0254	52.29	0.0292	49.69
Ketoconazole	8	1.00E-06	-6.00	0.0789	92.50	0.0575	87.48	0.0454	93.64	0.0524	89.06
4-OH androstenedione	1	1.00E-06	-6.00	0.0030	3.54	0.0025	3.74	0.0020	4.19	0.0026	4.36
4-OH androstenedione	2	5.00E-07	-6.30	0.0058	6.77	0.0044	6.70	0.0038	7.75	0.0044	7.40
4-OH androstenedione	3	2.50E-07	-6.60	0.0116	13.60	0.0081	12.40	0.0072	14.80	0.0086	14.67
4-OH androstenedione	4	1.00E-07	-7.00	0.0237	27.79	0.0176	26.75	0.0148	30.42	0.0171	29.15
4-OH androstenedione	5	5.00E-08	-7.30	0.0399	46.78	0.0297	45.14	0.0242	49.86	0.0286	48.69
4-OH androstenedione	6	2.50E-08	-7.60	0.0332	38.96	0.0411	62.44	0.0343	70.72	0.0404	68.76
4-OH androstenedione	7	1.00E-08	-8.00	0.0729	85.44	0.0527	80.13	0.0432	88.97	0.0492	83.62
4-OH androstenedione	8	1.00E-09	-9.00	0.0846	99.23	0.0584	88.80	0.0499	102.75	0.0567	96.43

Table 13. Recombinant Aromatase Activity in the Presence of Various Test Chemicals.

Chemical	Level	Concentration (M)	Log(concentration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Econazole	1	1.00E-06	-6.00	0.0014	0.26	0.0014	0.19	0.0009	0.31	0.0011	0.28
Econazole	2	5.00E-07	-6.30	0.0023	0.41	0.0016	0.21	0.0016	0.53	0.0015	0.38
Econazole	3	2.50E-07	-6.60	0.0048	0.87	0.0042	0.55	0.0028	0.93	0.0033	0.84
Econazole	4	1.00E-07	-7.00	0.0138	2.50	0.0102	1.34	0.0071	2.30	0.0069	1.77
Econazole	5	5.00E-08	-7.30	0.0215	3.90	0.0204	2.68	0.0122	3.96	0.0141	3.62
Econazole	6	2.50E-08	-7.60	0.0435	7.89	0.0327	4.29	0.0306	9.98	0.0256	6.58
Econazole	7	1.00E-08	-8.00	0.1031	18.70	0.1004	13.20	0.0606	19.74	0.0631	16.24
Econazole	8	1.00E-09	-9.00	0.3940	71.46	0.5893	77.47	0.2512	81.84	0.2851	73.42
Genistein	1	1.00E-03	-3.00	0.2423	43.95	0.3424	45.01	0.1620	52.80	0.1619	41.71
Genistein	2	5.00E-04	-3.30	0.2983	54.11	0.5291	69.55	0.1903	62.00	0.1957	50.40
Genistein	3	2.50E-04	-3.60	0.4047	73.40	0.6409	84.24	0.2510	81.80	0.2804	72.21
Genistein	4	1.00E-04	-4.00	0.4875	88.42	0.6792	89.28	0.3004	97.90	0.3024	77.87
Genistein	5	5.00E-05	-4.30	0.4836	87.70	0.7720	101.48	0.3131	102.01	0.3738	96.27
Genistein	6	2.50E-05	-4.60	0.5436	98.58	0.8465	111.28	0.3085	100.51	0.3837	98.82
Genistein	7	1.00E-05	-5.00	0.5334	96.74	0.6406	84.20	0.3288	107.15	0.3880	99.92
Genistein	8	1.00E-06	-6.00	0.6050	109.72	0.7188	94.48	0.3043	99.15	0.4139	106.59

Chemical	Level	Concentration (M)	Log(concentration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Atrazine	1	1.00E-03	-3.00	0.4423	80.21	0.5360	70.45	0.2144	69.87	0.2763	71.16
Atrazine	2	1.00E-04	-4.00	0.5167	93.70	0.6788	89.23	0.2966	96.66	0.3895	100.31
Atrazine	3	1.00E-05	-5.00	0.5813	105.43	0.7655	100.63	0.3183	103.72	0.3758	96.77
Atrazine	4	1.00E-06	-6.00	0.5600	101.55	0.6320	83.08	0.2962	96.51	0.3814	98.23
Atrazine	5	1.00E-07	-7.00	0.5418	98.27	0.7069	92.92	0.3078	100.29	0.3732	96.11
Atrazine	6	1.00E-08	-8.00	0.5565	100.93	0.7243	95.22	0.3005	97.93	0.3979	102.48
Atrazine	7	1.00E-09	-9.00	0.5264	95.46	0.8338	109.60	0.2857	93.08	0.4115	105.98
bis(2-ethylhexyl)phthalate	1	1.00E-03	-3.00	0.4542	82.37	0.5829	76.62	0.2935	95.64	0.3822	98.43
bis(2-ethylhexyl)phthalate	2	1.00E-04	-4.00	0.5740	104.10	0.8447	111.05	0.3060	99.69	0.4055	104.44
bis(2-ethylhexyl)phthalate	3	1.00E-05	-5.00	0.5415	98.21	0.6084	79.97	0.3159	102.94	0.4330	111.51
bis(2-ethylhexyl)phthalate	4	1.00E-06	-6.00	0.5550	100.65	0.7357	96.71	0.3005	97.92	0.3738	96.25
bis(2-ethylhexyl)phthalate	5	1.00E-07	-7.00	0.5320	96.48	0.6432	84.54	0.2720	88.63	0.3672	94.56
bis(2-ethylhexyl)phthalate	6	1.00E-08	-8.00	0.5348	96.98	0.6658	87.52	0.2926	95.34	0.3479	89.60
bis(2-ethylhexyl)phthalate	7	1.00E-09	-9.00	0.5448	98.80	0.6621	87.04	0.2788	90.85	0.3584	92.31
Aminoglutethimide	1	1.00E-03	-3.00	0.0026	0.34	0.0024	0.47	0.0017	0.55	0.0021	0.63
Aminoglutethimide	2	1.00E-04	-4.00	0.0297	3.94	0.0273	5.35	0.0161	5.10	0.0208	6.20
Aminoglutethimide	3	5.00E-05	-4.30	0.0548	7.28	0.0454	8.91	0.0330	10.46	0.0361	10.79
Aminoglutethimide	4	2.50E-05	-4.60	0.1061	14.08	0.0960	18.85	0.0652	20.67	0.0560	16.71
Aminoglutethimide	5	1.00E-05	-5.00	0.2515	33.38	0.1700	33.35	0.1075	34.07	0.1071	31.97
Aminoglutethimide	6	1.00E-06	-6.00	0.6070	80.54	0.4007	78.62	0.2779	88.08	0.2830	84.48
Aminoglutethimide	7	1.00E-07	-7.00	0.7206	95.61	0.5079	99.67	0.3239	102.67	0.3304	98.60
Aminoglutethimide	8	1.00E-08	-8.00	0.7253	96.24	0.5162	101.28	0.3202	101.47	0.3495	104.32

Chemical	Level	Concentration (M)	Log(concentration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Chrysin	1	1.00E-03	-3.00	0.1507	20.00	0.1286	25.23	0.0790	25.04	0.0803	23.98
Chrysin	2	1.00E-04	-4.00	0.0655	8.69	0.0663	13.01	0.0646	20.47	0.0477	14.25
Chrysin	3	5.00E-05	-4.30	0.0600	7.97	0.0600	11.77	0.0504	15.97	0.0483	14.40
Chrysin	4	2.50E-05	-4.60	0.0776	10.30	0.0646	12.67	0.0480	15.22	0.0490	14.62
Chrysin	5	1.00E-05	-5.00	0.1975	26.21	0.1368	26.85	0.0997	31.61	0.0955	28.49
Chrysin	6	1.00E-06	-6.00	0.6060	80.41	0.3886	76.26	0.2467	78.19	0.2900	86.57
Chrysin	7	1.00E-07	-7.00	0.8014	106.34	0.5112	100.30	0.3176	100.65	0.3345	99.83
Chrysin	8	1.00E-08	-8.00	0.7909	104.95	0.5036	98.82	0.3303	104.69	0.3401	101.52
Nonylphenol	1	1.00E-03	-3.00	0.0001	0.01	0.0003	0.06	0.0001	0.03	0.0000	0.01
Nonylphenol	2	1.00E-04	-4.00	0.0044	0.58	0.0048	0.95	0.0025	0.78	0.0023	0.69
Nonylphenol	3	1.00E-05	-5.00	0.5447	72.27	0.3648	71.57	0.2642	83.72	0.2297	68.57
Nonylphenol	4	1.00E-06	-6.00	0.7642	101.41	0.5206	102.14	0.3299	104.55	0.3305	98.66
Nonylphenol	5	1.00E-07	-7.00	0.7246	96.15	0.5256	103.14	0.3090	97.92	0.3424	102.20
Nonylphenol	6	1.00E-08	-8.00	0.7361	97.67	0.5047	99.04	0.3130	99.21	0.3256	97.18
Nonylphenol	7	1.00E-09	-9.00	0.7326	97.21	0.4807	94.33	0.2988	94.70	0.3403	101.57
Lindane	1	1.00E-03	-3.00	0.5671	75.24	0.3961	77.73	0.2403	76.17	0.2659	79.37
Lindane	2	1.00E-04	-4.00	0.6349	84.25	0.4666	91.55	0.2622	83.10	0.2994	89.37
Lindane	3	1.00E-05	-5.00	0.6856	90.98	0.4820	94.57	0.2905	92.08	0.3078	91.87
Lindane	4	1.00E-06	-6.00	0.7034	93.33	0.4827	94.71	0.3045	96.51	0.3225	96.25
Lindane	5	1.00E-07	-7.00	0.7234	95.99	0.4934	96.81	0.2960	93.82	0.3361	100.31
Lindane	6	1.00E-08	-8.00	0.7397	98.15	0.5001	98.13	0.3156	100.01	0.3040	90.73
Lindane	7	1.00E-09	-9.00	0.7294	96.78	0.5065	99.38	0.3226	102.25	0.3057	91.23

Chemical	Level	Concentration (M)	Log(concentration)	Day 1		Day 2		Day 3		Day 4	
				Activity	% Full activity						
Dibenz(a, h)anthracene	1	1.00E-04	-4.00	0.6839	86.86	0.3218	86.60	0.2915	93.28	0.3482	87.61
Dibenz(a, h)anthracene	2	1.00E-05	-5.00	0.8088	102.73	0.3633	97.77	0.3191	102.11	0.3957	99.58
Dibenz(a, h)anthracene	3	1.00E-06	-6.00	0.7737	98.26	0.3441	92.60	0.3773	120.73	0.4113	103.50
Dibenz(a, h)anthracene	4	1.00E-07	-7.00	0.9105	115.65	0.3681	99.05	0.3308	105.84	0.4353	109.53
Dibenz(a, h)anthracene	5	1.00E-08	-8.00	0.9131	115.97	0.3547	95.46	0.3150	100.80	0.4178	105.14
Dibenz(a, h)anthracene	6	1.00E-09	-9.00	0.8045	102.19	0.3430	92.31	0.2928	93.70	0.3416	85.95
Ketoconazole	1	8.00E-04	-3.10	0.0006	0.08	0.0003	0.10	-0.0001	-0.03	0.0002	0.08
Ketoconazole	2	5.00E-04	-3.30	0.0042	0.53	0.0026	0.79	0.0002	0.06	0.0018	0.58
Ketoconazole	3	2.50E-04	-3.60	0.0083	1.05	0.0025	0.74	0.0010	0.37	0.0019	0.60
Ketoconazole	4	1.00E-04	-4.00	0.0280	3.54	0.0219	6.59	0.0147	5.47	0.0180	5.67
Ketoconazole	5	5.00E-05	-4.30	0.0655	8.29	0.0435	13.07	0.0342	12.72	0.0367	11.57
Ketoconazole	6	2.50E-05	-4.60	0.0958	12.12	0.0772	23.24	0.0600	22.31	0.0805	25.40
Ketoconazole	7	1.00E-05	-5.00	0.2439	30.86	0.1433	43.12	0.1194	44.40	0.1308	41.28
Ketoconazole	8	1.00E-06	-6.00	0.6739	85.27	0.2644	79.55	0.2390	88.84	0.2955	93.25
4-OH androstenedione	1	1.00E-06	-6.00	0.0251	3.18	0.0298	8.97	0.0201	7.46	0.0397	12.54
4-OH androstenedione	2	5.00E-07	-6.30	0.0465	5.89	0.0443	13.32	0.0362	13.46	0.0518	16.36
4-OH androstenedione	3	2.50E-07	-6.60	0.0789	9.98	0.0648	19.49	0.0597	22.21	0.1010	31.87
4-OH androstenedione	4	1.00E-07	-7.00	0.1413	17.88	0.1246	37.50	0.0964	35.84	0.1627	51.35
4-OH androstenedione	5	5.00E-08	-7.30	0.2300	29.10	0.1587	47.75	0.1454	54.04	0.2178	68.71
4-OH androstenedione	6	2.50E-08	-7.60	0.3845	48.65	0.1962	59.02	0.1914	71.17	0.2467	77.85
4-OH androstenedione	7	1.00E-08	-8.00	0.5280	66.81	0.2541	76.43	0.2550	94.81	0.2800	88.35
4-OH androstenedione	8	1.00E-09	-9.00	0.6729	85.15	0.2739	82.39	0.2970	110.40	0.3036	95.79

See files Figure 11.wpd and Figure 12.wpd for response curves of placental and recombinant assay data, respectively.

IC₅₀ and slope estimates are presented in Tables 14 and 15 for the placental and recombinant assays, respectively. Side by side comparisons (by test substance and microsome type) of IC₅₀ and slope are presented in Tables 16 and 17, respectively.

Table 14. IC₅₀ and Slope Estimates by Chemical for the Placental Assay

Chemical	IC50 Estimates				Slope Estimates			
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4
4-OH androstenedione	3.230E-08	3.926E-08	5.188E-08	4.767E-08	-0.952	-1.002	-1.188	-1.089
Aminoglutethimide	1.996E-06	7.696E-06	7.540E-06	9.196E-06	-1.142	-0.961	-1.009	-1.184
Atrazine	2.412E-02	1.884E-03	.	.	-0.687	-0.783	.	.
Chrysins	1.582E-06	6.110E-06	7.098E-06	6.191E-06	-1.119	-0.877	-1.122	-0.830
Dibenz(a, h)anthracene
Econazole	1.943E-09	6.473E-10	2.837E-09	2.124E-09	-1.138	-1.132	-1.065	-1.003
Genistein	2.386E-03	4.140E-04	3.890E-03	2.297E-03	-0.619	-0.973	-0.791	-0.504
Ketoconazole	1.166E-05	1.069E-05	1.116E-05	9.934E-06	-1.070	-0.969	-1.109	-0.972
Lindane	3.665E-02	.	8.972E-02	4.492E-01	-0.337	.	-0.548	-0.232
Nonylphenol	1.691E-05	.	.	7.955E-05	-1.227	.	.	-0.955
bis(2-ethylhexyl)phthalate	.	3.102E-03	.	.	.	-2.000	.	.

Table 15 IC₅₀ and Slope Estimates by Chemical for the Recombinant Assay

Table 16 IC50 Estimates by Chemical and Microsome Type

Chemical	Microsome					Geo Std	Dev
	Type	Rep 1	Rep 2	Rep 3	Rep 4	Geo Mean	
4-OH androstenedione	Placental	3.230E-08	3.926E-08	5.188E-08	4.767E-08	4.208E-08	1.23550
4-OH androstenedione	Recombinant	1.988E-08*	4.145E-08	6.461E-08	1.055E-07	4.868E-08	2.03064
Aminoglutethimide	Placental	1.996E-06*	7.696E-06	7.540E-06	9.196E-06	5.713E-06	2.02745
Aminoglutethimide	Recombinant	4.433E-06	4.576E-06*	6.017E-06	5.057E-06	4.984E-06	1.14735
Atrazine ^a	Placental	2.412E-02	1.884E-03	.	.	6.741E-03	6.06829
Atrazine ^a	Recombinant	5.890E-03	2.590E-02*	2.048E-03	.	6.786E-03	3.57692
Chrysins	Placental	1.582E-06*	6.110E-06	7.098E-06	6.191E-06	4.540E-06	2.02569
Chrysins	Recombinant	3.894E-06	3.581E-06*	4.404E-06	4.902E-06	4.166E-06	1.14777
Dibenz(a, h)anthracene ^a	Placental
Dibenz(a, h)anthracene ^a	Recombinant	.	.	.	3.138E-04	3.138E-04	.
Econazole	Placental	1.943E-09	6.473E-10*	2.837E-09	2.124E-09	1.659E-09	1.91168
Econazole	Recombinant	2.418E-09	2.502E-09	3.374E-09	2.425E-09	2.653E-09	1.17490
Genistein ^a	Placental	2.386E-03	4.140E-04*	3.890E-03	2.297E-03	1.724E-03	2.66613
Genistein	Recombinant	7.000E-04	8.799E-04	9.467E-04	6.057E-04	7.709E-04	1.22865
Ketoconazole	Placental	1.166E-05	1.069E-05	1.116E-05	9.934E-06	1.084E-05	1.07060
Ketoconazole	Recombinant	4.795E-06*	6.146E-06	7.867E-06	8.087E-06	6.580E-06	1.27689
Lindane ^a	Placental	3.665E-0*2	.	8.972E-02	4.492E-01	1.139E-01	3.56059
Lindane ^a	Recombinant	1.410E-01	6.920E-02	6.746E-02	.	8.700E-02	1.51994
Nonylphenol ^a	Placental	1.691E-05*	.	.	7.955E-05	3.668E-05	2.98875
Nonylphenol ^a	Recombinant	.	.	.	1.644E-05	1.644E-05	.
bis(2-ethylhexyl)phthalate ^a	Placental	.	3.102E-03	.	.	3.102E-03	.
bis(2-ethylhexyl)phthalate ^a	Recombinant

* High positive control activity indicative of possible substrate solution prep error may have affected these IC50 results.

^a Response curves did not pass through 50% of control activity. IC50 estimates are questionable.

Table 17 Slope Estimates by Chemical and Microsome Type

Chemical	Microsome Type						
		Rep 1	Rep 2	Rep 3	Rep 4	Mean	Std Dev
4-OH androstenedione	Placental	-0.952	-1.002	-1.188	-1.089	-1.057	0.10372
4-OH androstenedione	Recombinant	-0.847	-0.686	-1.068	-0.916	-0.879	0.15824
Aminoglutethimide	Placental	-1.142	-0.961	-1.009	-1.184	-1.074	0.10586
Aminoglutethimide	Recombinant	-0.973	-0.909	-1.061	-1.005	-0.987	0.06363
Atrazine	Placental	-0.687	-0.783	.	.	-0.735	0.06837
Atrazine	Recombinant	-0.779	-0.312	-1.171	.	-0.754	0.42988
Chrysins	Placental	-1.119	-0.877	-1.122	-0.830	-0.987	0.15508
Chrysins	Recombinant	-1.113	-0.937	-0.887	-1.068	-1.001	0.10662
Dibenz(a, h)anthracene	Placental
Dibenz(a, h)anthracene	Recombinant	.	.	.	-1.710	-1.710	.
Econazole	Placental	-1.138	-1.132	-1.065	-1.003	-1.085	0.06341
Econazole	Recombinant	-1.040	-1.341	-1.210	-1.140	-1.183	0.12651
Genistein	Placental	-0.619	-0.973	-0.791	-0.504	-0.722	0.20463
Genistein	Recombinant	-0.926	-1.337	-1.200	-0.946	-1.102	0.20020
Ketoconazole	Placental	-1.070	-0.969	-1.109	-0.972	-1.030	0.07054
Ketoconazole	Recombinant	-1.119	-0.862	-1.070	-1.116	-1.042	0.12172
Lindane	Placental	-0.337	.	-0.548	-0.232	-0.372	0.16127
Lindane	Recombinant	-0.229	-0.314	-0.266	.	-0.269	0.04245
Nonylphenol	Placental	-1.227	.	.	-0.955	-1.091	0.19232
Nonylphenol	Recombinant	.	.	.	-1.568	-1.568	.
bis(2-ethylhexyl)phthalate	Placental	.	-2.000	.	.	-2.000	.
bis(2-ethylhexyl)phthalate	Recombinant

